THE VELIGER
© CMS, Inc., 2004

The Veliger 47(2):141–152 (April 30, 2004)

Gonad Structure and Gamete Morphology of the Eastern South Pacific Chiton *Acanthopleura echinata* Barnes, 1824

CARLOS F. GAYMER,[1,2*] CHITA GUISADO,[1,3] KATHERINA B. BROKORDT,[1,4] AND JOHN H. HIMMELMAN[5]

[1] Departamento de Biología Marina, Center for Advanced Studies in Arid Zones, Universidad Católica del Norte, Casilla 117, Coquimbo, Chile

[2] Departamento de Ecología, Center for Advanced Studies in Ecology and Biodiversity, P. Universidad Católica de Chile, Alameda 340, Casilla 114-D, Santiago, Chile

[3] Facultad de Ciencias del Mar, Universidad de Valparaíso, Av. Borgoño s/n, Montemar, Viña del Mar, Chile

[4] Departamento de Ecología, P. Universidad Católica de Chile, Alameda 340, Casilla 114-D, Santiago, Chile

[5] Département de Biologie, Université Laval, Québec, Canada G1K 7P4

Abstract. We examined gonad structure and gamete morphology of *Acanthopleura echinata,* one of the largest and most abundant chitons along the coastline of the eastern South Pacific. Observations of sections of the gonads of adult *A. echinata* revealed that the wall of the testis and ovary is constituted of a muscular-connective tissue layer and that this wall is folded toward the lumen, forming numerous tissue plates. In the testis, the germinal epithelium, which forms a continuous covering on the plates and on the lateral and ventral walls, gives rise to layers of spermatogonia, then spermatocytes, and finally spermatids. In the ovary, the germinal epithelium is found on the lateral and ventral walls of the ovary, but not on the tissue plates, and previtellogenic and vitellogenic oocytes are attached to the tissue plates. Mature gametes accumulate in the lumen of both the testis and ovary. Numerous simple and externally ciliated blood vessels run from the dorsal aorta to the tissue plates and, in addition to supplying blood to the gonad tissues, may aid with the expulsion of gametes during spawning. We used light and scanning electronic microscopy to examine gametes that were obtained from a spontaneous spawning and by stripping the gonads. Spawned oocytes are in the first meiotic prophase and measure 345 μm in diameter. They are filled with green yolk, and are surrounded by a type III chorion, with numerous cylindrical projections that expand distally. The spermatozoa are primitive and possess an 8-μm-long triangular head, with an anterior filament.

INTRODUCTION

Most chitons have separate sexes that cannot be distinguished externally except in a few species where the males and females differ in color (Hyman, 1967). Chitons have a single gonad located midway under the shell valves and which differs in color between males and females (Pearse, 1979). The gonad is saclike in structure with the inner wall folded toward the lumen, forming numerous tissue plates. Developing gametes are attached to the plates, and the ripe gametes accumulate in the lumen. The gametes are spawned through two gonoducts which exit into the left and right pallial grooves at the position between the sixth and seventh shell valves (Hyman, 1967; Selwood, 1968; Pearse, 1979). The spermatozoa are carried away by the exhalant currents leaving the pallial grooves, whereas oocytes are usually laid in masses or liberated in gelatinous strings secreted by the oviduct (Hyman, 1967; Pearse, 1979). Fertilization takes place externally or within the female's mantle cavity (Hyman, 1967).

The oocytes of chitons can be classified into three categories according to the degree of development: (1) previtellogenic oocytes, which are pear-shaped and attached to the ovary wall; (2) vitellogenic oocytes that are spherical and still attached to the ovary wall (at this stage dense masses of yolk accumulate in the cytoplasm, and the follicle cells surrounding each oocyte increase in volume and change in shape); and (3) fully developed (mature) oocytes that are filled with green yolk and are surrounded by a chorion which is made up of numerous differently shaped structures. The chorion is thought to be produced either by the follicle cells (Anderson, 1969) or by oocyte microvilli (Pearse, 1979). According to the shape of the chorion, mature oocytes fall into three categories (Risbec, 1946): (1) smooth, as observed in *Lepidopleurus asellus* (Gmelin, 1791), by Christiansen (1954); (2) with numerous cupules, lobules, or plates, as observed in *Mopalia muscosa* (Gould, 1846) by Anderson (1969), *Tonicella lineata* (Wood, 1815) by Buckland-Nicks et al. (1988a), *Lepidochitona dentiens* (Gould,

* Corresponding author: Telephone: 56 (51) 209809; Fax: 56 (51) 209803; e-mail: cgaymer@ucn.cl; cgaymer@bio.puc.cl

1846) by Buckland-Nicks (1993) and *Lepidochitona fernaldi* Eernisse, 1986, by Buckland-Nicks & Eernisse (1993); and (3) with numerous slender projections or spines, as observed in *Ischnochiton albus* (Linnaeus, 1767) by Lyngnes (1924) and *Chiton cumingsii* Frembly, 1827, by Schweikart (1904). When vitellogenesis is completed, the follicle cells degenerate and are shed from the mature oocyte (Pearse, 1979; Buckland-Nicks & Eernisse, 1993).

Chitons were originally thought to have primitive spermatozoa with three regions, the head (with an anterior filament), mid-piece and flagellum (Hyman, 1967; Pearse, 1979; Dohmen, 1983; Al-Hajj, 1987; Hodgson et al., 1988). However, Russell-Pinto et al. (1983, 1984) indicate that some chitons have modified spermatozoa because of the lack of an acrosome, and the great number and variable position of mitochondria observed in some species. The hypothesis that some chitons have modified spermatozoa is not widely accepted (Hodgson et al., 1988). Numerous studies indicate that the anterior filament is just an extension of the nucleus and that it does not have an acrosome (Pearse, 1979; Pearse & Woollacott, 1979; Russell-Pinto et al., 1983; Russell-Pinto et al., 1984; Hodgson et al., 1988). However, the presence of an acrosome may depend on the species as recent studies indicate the presence of a granule at the distal end of the anterior filament in a number of chiton species. This granule is involved in fertilization and is formed by the fusion of lysosomic vesicles produced by the Golgi complex (Hodgson et al., 1988; Buckland-Nicks et al., 1988a, b, 1990; Buckland-Nicks & Eernisse, 1993; Pashchenko & Drozdov, 1998).

In the eastern South Pacific, especially in Chile, chitons have been commercially harvested in recent years for human consumption of the foot (Osorio, 1989; Sernapesca, 2000). A better knowledge of their biology is required so that adequate strategies can be developed for managing this resource and to develop techniques for the culture of chitons. The present study examines two aspects of the reproductive biology of the dioecious chiton *Acanthopleura echinata,* the structure of the gonad and the morphology of the gametes. *A. echinata* is abundant in the low intertidal and upper subtidal zones in very exposed rocky shores in Peru and northern and central Chile (Boudet, 1945; Leloup, 1956; Guiller, 1959; Otaíza & Santelices, 1985; Otaíza, 1986; Santelices et al., 1986). *A. echinata* is one of the most heavily exploited chitons in Chile and Peru because of its large size (~ 10–15 cm in length), high abundance, and wide distribution (Boudet, 1945; Leloup, 1956; Guiller, 1959; Osorio, 1989; Otaíza & Santelices, 1985; Otaíza, 1986; Sernapesca, 2000).

MATERIALS AND METHODS

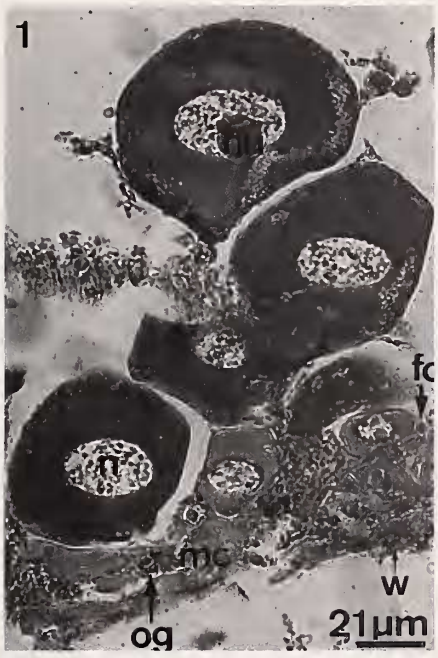
We examined the structure of the gonad of *Acanthopleura echinata* from 20 male and female adults collected from the rocky intertidal zone at Lagunillas (29°59′S: 71°22′W), northern Chile, during each of five periods, in January, April, July, October, and December 1993. Gamete morphology was studied from gametes obtained from 10 adults collected at the same place during each of three periods, in December 1993, January 1994, and February 1994. The latter individuals were maintained with a supply of *Ulva* sp. as food in 40-l aquaria in a wet laboratory at Universidad Catolica del Norte, Chile, so that the gametes could be obtained.

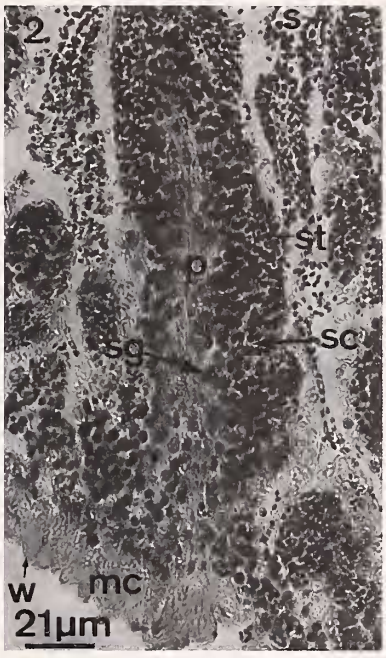
To study gonad morphology, the gonad of each chiton was removed and transferred to Bouin-Hollande's fixative. Later, the gonad was washed with tap water and divided into three portions (anterior, middle and posterior), and each was processed separately using the standard embedding technique as described by Luna (1968). Thirty 5-μm microtome sections were cut from each gonad portion, 20 of which were stained with hematoxalin-eosin and 10 with the Mallory trichrome stain (Luna, 1968). The sections were observed using light microscopy.

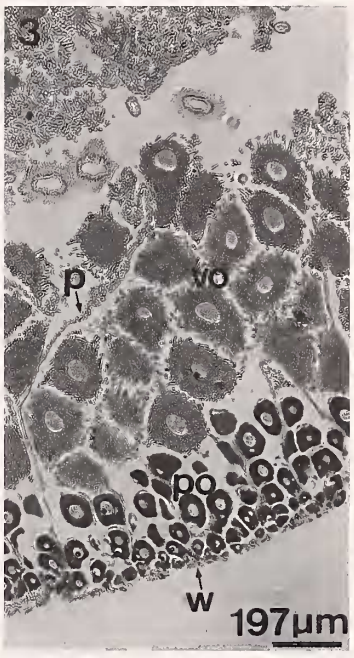
Gametes were obtained from a single spontaneous spawning that occurred in the field in December 1993 (no chitons spawned in the laboratory), and also by stripping the gonads of the chitons kept in the laboratory. In both

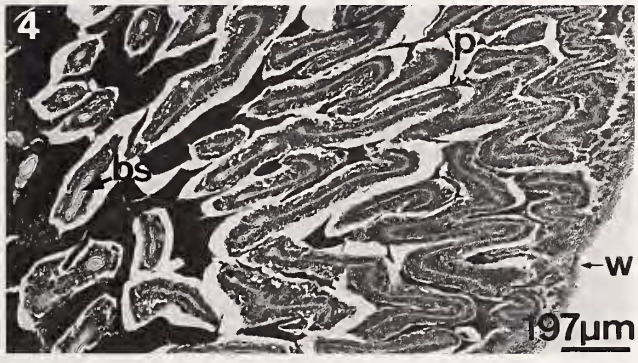
→

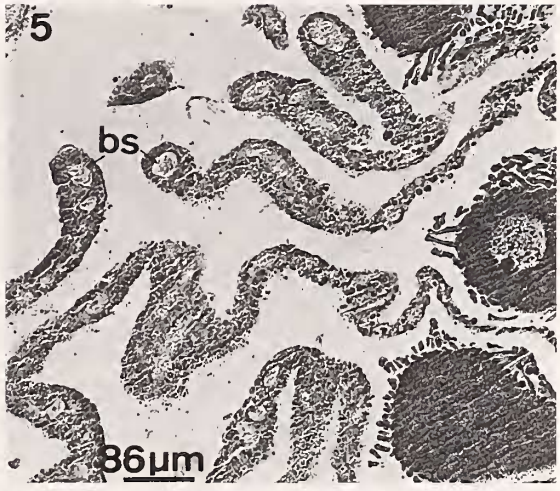
Figures 1–7. A section of the ovary of *Acanthopleura echinata* (stained with Mallory) showing previtellogenic oocytes, each with large nucleus (n) and nucleolus (nu), the gonad wall (w), muscular-connective tissue (mc), follicle cells (fc) and oogonia (og). Figure 2. Section of the testis of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing the gonad wall (w), muscular-connective tissue (mc), tissue plate (p) and layers of spermatogonia (sg), spermatocytes (sc), spermatids (st) and spermatozoa (s). Figure 3. Section of the ovary of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing a general view of the gonad wall (w), tissue plates (p), and previtellogenic (po) and vitellogenic (vo) oocytes. Figure 4. Section of the testis of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing a general view of the gonad wall (w) and numerous tissue plates (p) with blood sinuses (bs). Figure 5. Section of recently spawned ovary of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing numerous tissue plates with blood sinuses (bs). Figure 6. Section of the ovary of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing a connection between a non-ciliated vessel (top) and a tissue plate (bottom), and a blood sinus (bs) between the two layers of columnar cells (cc). Figure 7. Section of ciliated (to the left) and non-ciliated vessels (on the right), each internally lined by muscular-connective tissue (mc), within the lumen of a gonad of *Acanthopleura echinata* (stained with hematoxylin-eosin).

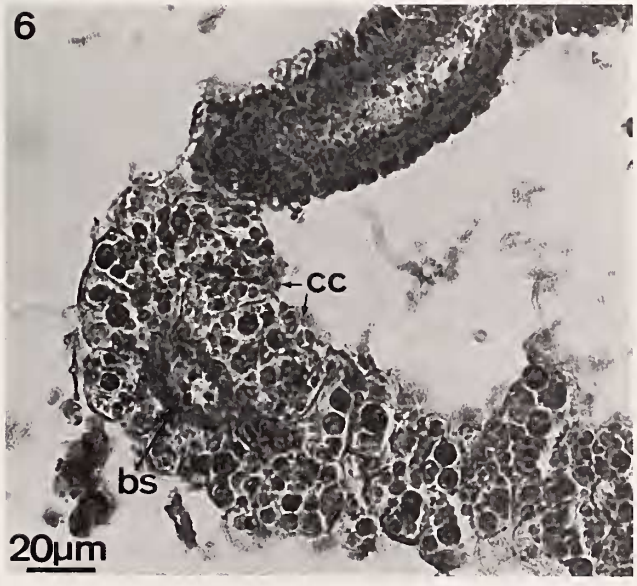

1
n
n
fc
mc
w
og
21µm

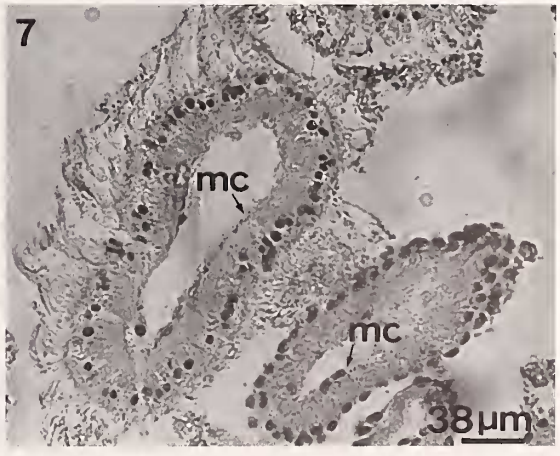

2
s
st
o
sg
sc
w
mc
21µm


3
p
vo
po
w
197µm


4
p
bs
w
197µm


5
bs
86µm


6
cc
bs
20µm


7
mc
mc
38µm

cases gametes were observed using light microscopy and also using scanning electronic microscopy (a JEOL JSM T-300 scanning electron microscope). Gametes for the electronic microscopy studies were fixed with 2% glutaraldehyde in seawater, subsequently washed in seawater, and then dehydrated using a graded series of ethanol. Finally, the samples were critical point dried and sputter-coated with gold. A sub-sample of spermatozoa was stained with 0.025% Acridine Orange and studied using fluorescence microscopy. We further studied gamete morphology from observations of the gametes in the histological sections of the gonads.

RESULTS

Gonad Structure

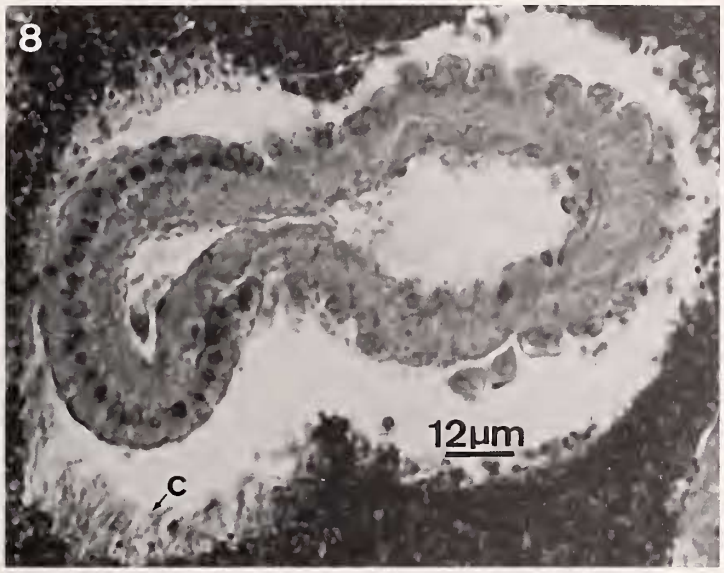
The ovaries of *Acanthopleura echinata* were dark green in color and the testes light brown. The gonad was attached by muscular tissue and connective fibers to the dorsal wall and dorsal aorta of the chiton. The outer gonad wall was made of layers of smooth muscle cells and connective fibers (the muscular-connective tissue layer) that stained light blue with Mallory dye (Figures 1, 2). In the lateral and ventral portions of the gonad, the wall was internally lined by a germinal epithelium composed by wall cells, germinal cells, and recently differentiated follicle cells and oogonia or spermatogonia (Figures 1, 2). In the dorso-lateral portion of the gonad, the germinal epithelium was interrupted and replaced first by irregular-shaped cells and then by an epithelium with cube-shaped cells. The cube-shaped cells were ciliated in the dorsal portion of the gonad and were continuous with the internal epithelium of the gonoducts.

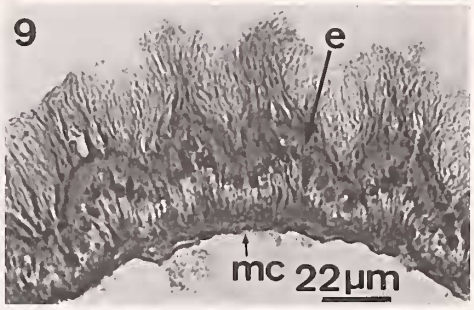
The numerous tissue plates (or folds), which extended from the ventral and ventro-lateral regions of the gonad wall toward the lumen (Figures 2, 3, 4), were composed of two layers of flattened cells and supported by muscular-connective tissue. Gametes in different stages of development were attached to these tissue plates. Between the two layers of flattened cells, there were numerous blood sinuses that received blood from vessels from the dorsal aorta (Figures 5, 6). These sinuses probably supplied materials to the developing gametes during maturation (e.g., vitellogenesis). We observed two types of blood vessels within the lumen of the gonads: (1) externally ciliated vessels (ciliated tubules *sensu* Selwood 1968) coming directly from the dorsal aorta, and (2) non-ciliated vessels, which were smaller in diameter and derived from the ciliated vessels (Figure 7). The non-ciliated vessels were connected to the tissue plates and likely supplied blood to the blood sinuses (Figure 6). Columnar cells made up the outer portion of the ciliated vessels, whereas irregular cells made up the outer portion of the non-ciliated vessels (Figure 8). Internally, both types of vessels were lined by a circular muscle layer and connective tissue, probably collagen (Figures 7, 8). During spawning, the ciliated vessels showed a morphological change, as the columnar epithelium became undulated, probably due to contraction of the internal muscular layer (Figure 9).

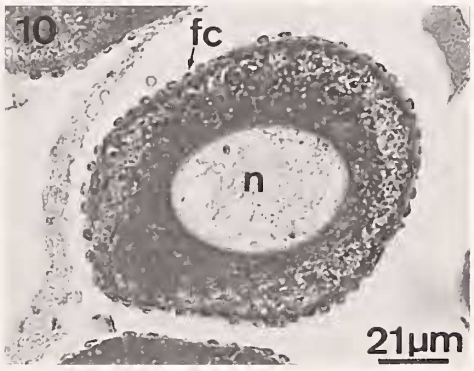
Ovary. The previtellogenic oocytes were pear-shaped, attached to the ovary wall and the base of the tissue plates, and covered with follicle cells. They measured ~ 60 μm in diameter, had a large nucleus with a large nucleolus, and a cytoplasm that strongly stained with hematoxylin (Figures 1, 3, 10). The vitellogenic oocytes measured ~140 μm and were attached to the mid- and distal regions of the tissue plates. The cytoplasm of the vitellogenic oocytes was strongly stained with eosin and was filled with yolk granules (Figures 3, 11). The formation of the chorion began at the vitellogenic stage. First, chorion projections began to form as small structures, each associated with a follicle cell (Figure 11). As vitellogenic oocytes approached the distal edge of the tissue plates, they increased in diameter, and the chorion projections elongated giving the oocytes a spiny appearance (Figure 3). A follicle cell was still associated with each projection (Figure 12). The mature oocytes were free in the ovary lumen and were completely surrounded by a chorion with numerous cylindrical projections with ex-

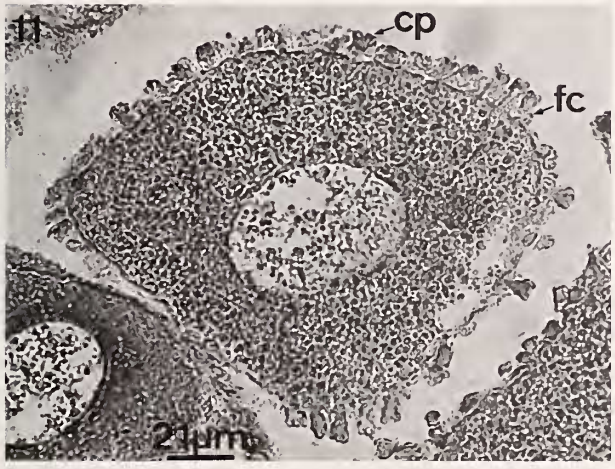
→

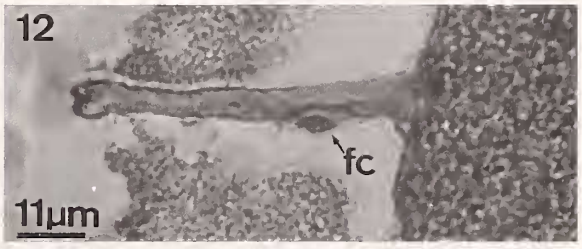
Figures 8–15. Section of the testis of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing a connection between a ciliated vessel (to the left) with detached cilia (c) and a non-ciliated vessel (on the right). Figure 9. Section of a ciliated vessel within a gonad of *Acanthopleura echinata* during spawning, showing the undulated epithelium (e) and the internal muscular-connective tissue layer (mc) (stained with hematoxylin-eosin). Figure 10. Section of a previtellogenic oocyte of *Acanthopleura echinata* covered with follicle cells (fc) and showing a large nucleus (n) (stained with hematoxylin-eosin). Figure 11. Section of an early vitellogenic oocyte of *Acanthopleura echinata* showing the beginning of the formation of the projections of the chorion (cp) and follicle cells (fc) associated with the projections (stained with Mallory). Figure 12. Section of a chorion projection in a late vitellogenic oocyte of *Acanthopleura echinata* showing a follicle cell (fc) at the side of the projection (stained with hematoxylin-eosin). Figure 13. Section of the ovary of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing mature oocytes (mo), each with a fully developed chorion, free in the lumen. Figure 14. Section of a mature oocyte of *Acanthopleura echinata* (stained with hematoxylin-eosin). Figure 15. Section of a chorion projection of a mature oocyte of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing the expanded distal portion of the projection and the follicle cell (fc) at the side of the projection.

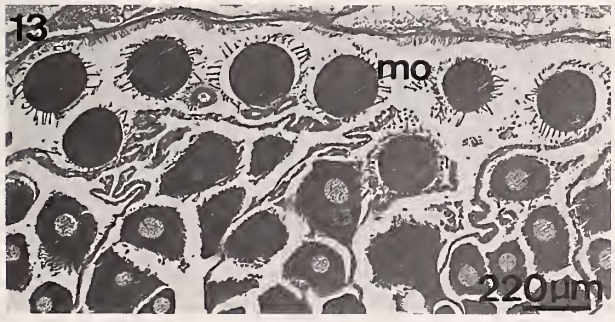

8
12µm
c

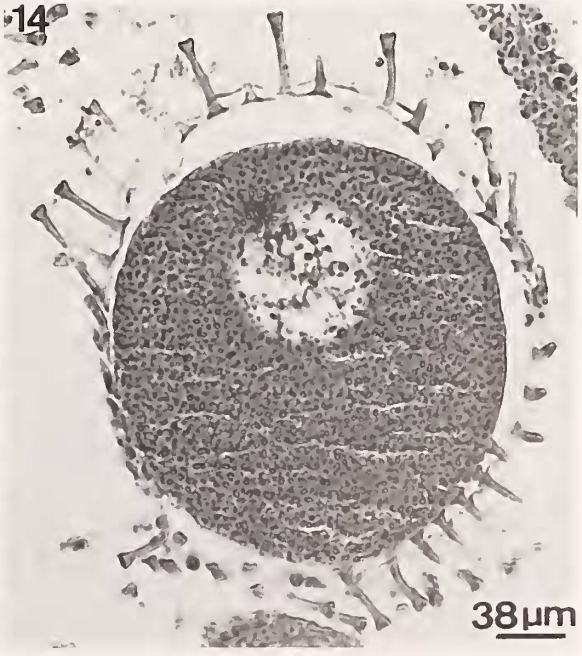

9
e
mc
22µm


10
fc
n
21µm


11
cp
fc
21µm


12
fc
11µm


13
mo
220µm


14
38µm

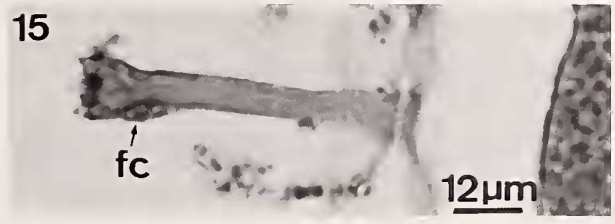

15
fc
12µm

Table 1

Acanthopleura echinata. Size of mature oocytes and spermatozoa.

		$\bar{X}$	SD	n
Oocytes	Diameter	345.0	5.00	30
	Diameter excluding the chorion	225.0	5.00	30
Spermatozoa	Head length	8.0	0.00	60
	Head width	1.8	0.03	60
	Mid piece length	1.0	0.0	60
	Mid piece width	1.9	0.02	60
	Flagellum length	23.0	0.50	60

panded distal portions (Figures 13, 14). In some cases follicle cells were still observed along the sides or distal edges of the projections, but in most cases they had disappeared (Figure 15).

Toward the distal edge of the tissue plates, the flattened cells increased in height and became columnar, with the cytoplasm filled with granules (Figure 6). In the region where the oviducts left the ovary, the ciliated cubic cells lining the dorsal wall of the gonad elongated to form a ciliated-columnar epithelium in the first portion of the oviducts (Figure 16). The columnar cells elongated progressively, but after a short distance abruptly changed to a glandular, pseudostratified epithelium with extremely high ciliated sac-shaped cells (Figure 16). The cytoplasm of the glandular cells (secretory) was filled with mucus and granular material (Figure 17). The distal ends of the glandular cells were separated from one another by extremely slender cells that had wedge-shaped distal ends (wedge cells) (Figure 17). Two large sacs of mucus, supported by connective fibers, were associated with the dorsal wall of the ovary and oviducts (slime sacs, *sensu* Hyman 1967).

Testis. The tissue plates were much more numerous in the male than in the female gonad (Figure 4). Moreover, the flattened cells were present all along the plates and were covered by germinal epithelium which was similar to that found in the gonad wall (Figure 2). As in the ovary, blood sinuses were present between the two layers of flattened cells (Figure 4).

A layer of large polygonal spermatogonia covered the germinal epithelium. Toward the gonad lumen, we observed layers of spermatocytes and small and slender spermatids (Figure 2). Spermatozoa accumulated in the testis lumen and formed an homogeneous mass that represented ~ 70% of the volume of the fully mature testis (Figures 2, 4).

The ciliated cubic epithelium lining the dorsal wall of the testis showed no morphological changes as the sperm ducts left the testis and was continuous with the ciliated cubic epithelium that lined the sperm ducts.

Gamete Morphology

The mature oocytes of *Acanthopleura echinata* measured 345 μm in diameter (SD = 5) (Table 1) and had a large amount of dark green yolk (Figure 18). A large germinal vesicle measured about ~ 1/4 of the diameter of the oocyte's cytoplasm (Figure 14). The chorion projections measured 60 μm (SD = 0) in length and their distal portions were expanded into crowns with six to seven spikes (Figure 19). The distal portions of chorion projections in immature oocytes had partially expanded crowns with six to seven rounded protuberances instead of spikes (Figures 20, 21, 22).

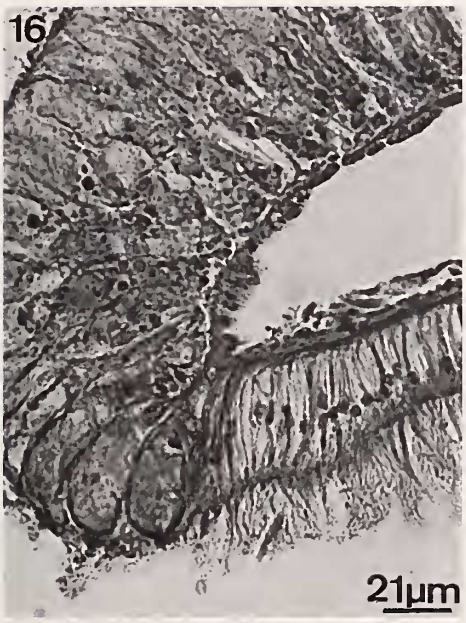
The head of the spermatozoa of *Acanthopleura echinata* was triangular, measured 8 μm (SD = 0) in length, and had an anterior filament (Table 1, Figures 23, 24). The mid-piece contained large mitochondria and was mainly in the posterior end of the head (Figure 24) (in some cases the mid piece was in postero-lateral position). The flagellum measured 23 μm (SD = 0.5) in length (Table 1, Figure 23). The entire head (including anterior filament) appeared fluorescent when stained with Acridine Orange. In the histological sections of the testes the head was strongly stained with hematoxylin and the mid-piece with eosin.

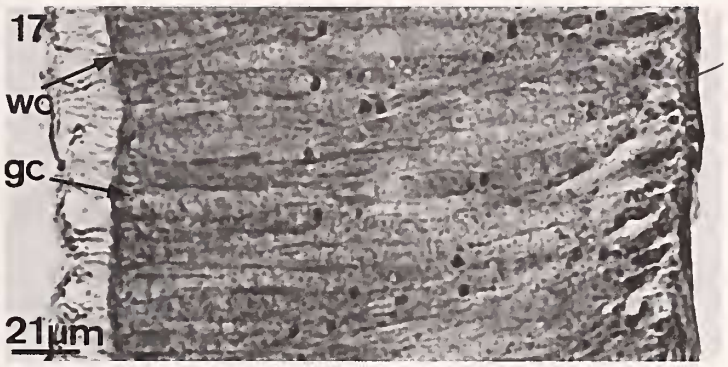
DISCUSSION

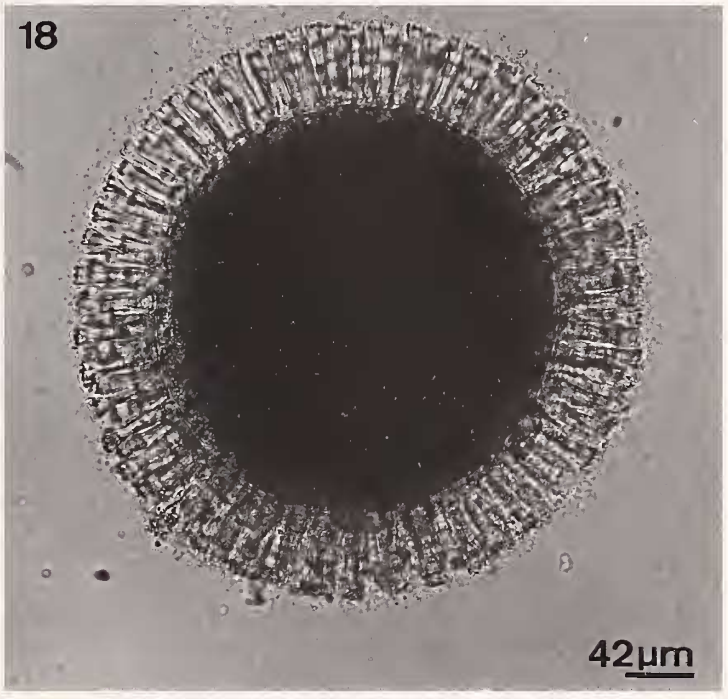
The structure of the gonad of *Acanthopleura echinata* generally resembles that of other species of chitons (Plate, 1897; Hyman, 1967; Selwood, 1968; Anderson, 1969; Pearse, 1979; Yoshioka, 1986). The presence of tissue plates formed by infolding of ventral and lateral gonad

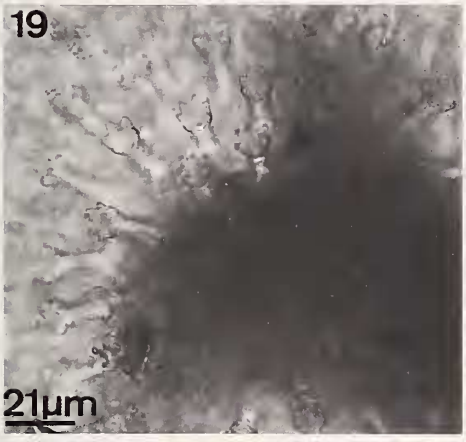
→

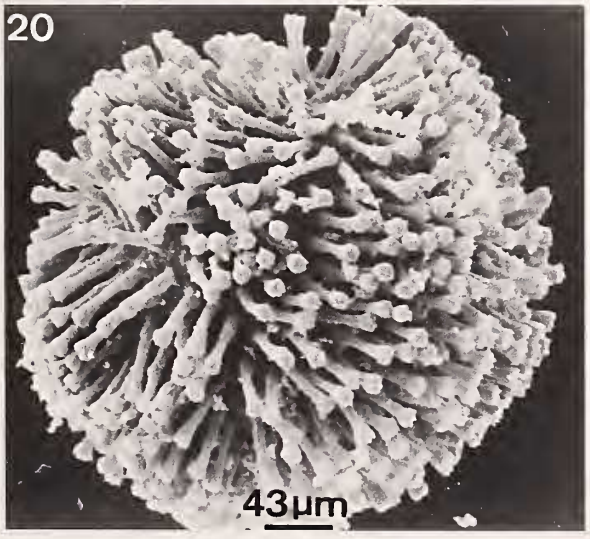
Figures 16–21. Section of the oviduct of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing the ciliated columnar epithelium of the first portion of the oviduct (on the right) and the abrupt change to the glandular pseudostratified epithelium with ciliated sac-shaped cells (to the left). Figure 17. Section of the oviduct of *Acanthopleura echinata* (stained with hematoxylin-eosin) showing the glandular pseudostratified epithelium, with identified glandular (gc) and wedge (wc) cells. Figure 18. A spontaneously spawned oocyte of *Acanthopleura echinata*. Figure 19. Detail of the chorion of a spontaneously spawned oocyte of *Acanthopleura echinata* showing distal portions of projections that are expanded into crowns with six to seven spikes. Figure 20. Scanning electron micrograph of an immature oocyte of *Acanthopleura echinata* that was striped from an ovary. Figure 21. Scanning electron micrograph of a portion of the chorion of an immature oocyte of *Acanthopleura echinata* showing rounded distal portions of projections.

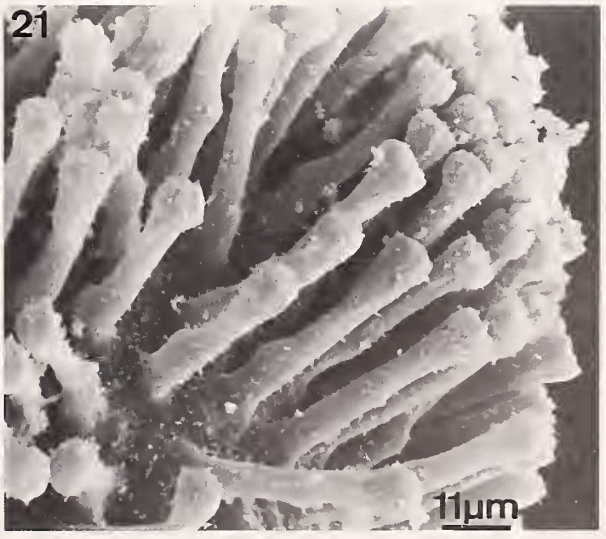

16
21µm

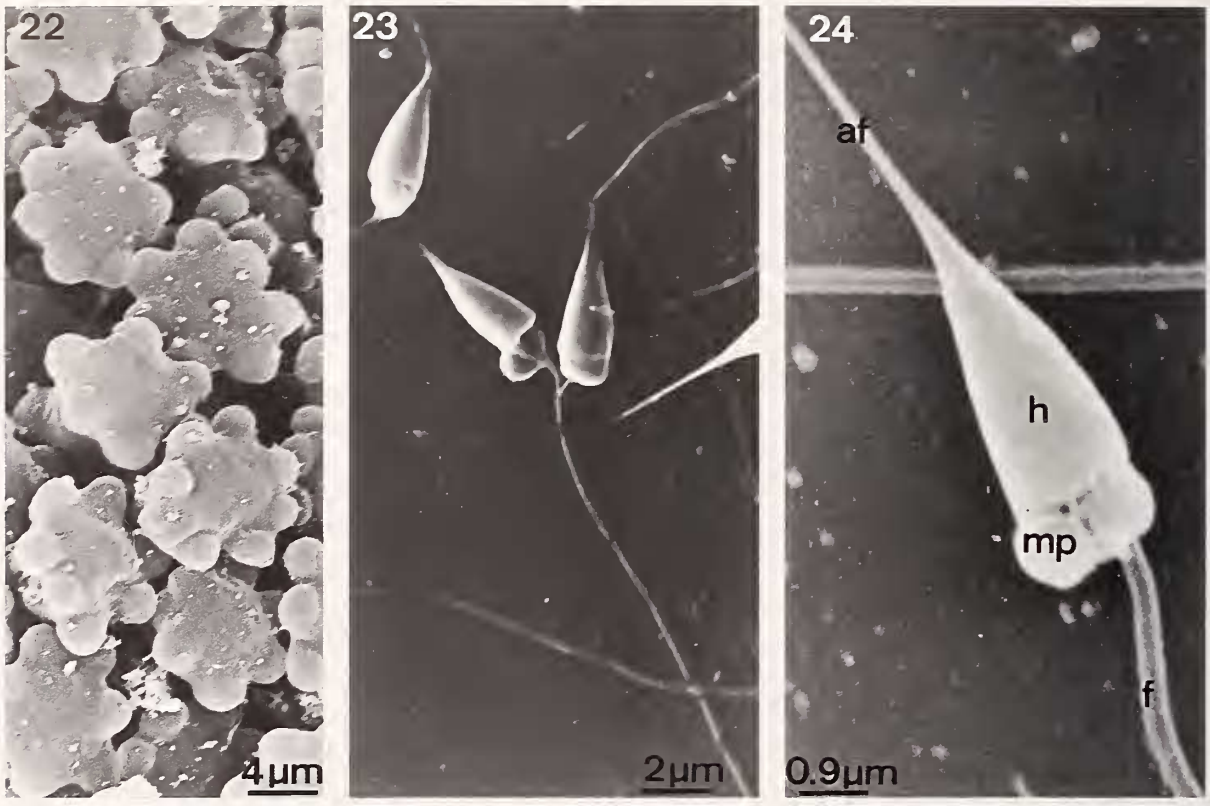

17
wc
gc
21µm


18
42µm


19
21µm


20
43µm


21
11µm



Figures 22–24. Scanning electron micrograph of the distal portion of chorion projections of an inmature oocyte of *Acanthopleura echinata* showing partially expanded distal portions of projections with six to seven rounded protuberances (rather than spikes). Figure 23. Scanning electron micrograph of spermatozoa of *Acanthopleura echinata*. Figure 24. Scanning electron micrograph of a spermatozoon of *Acanthopleura echinata* showing the head (h), mid-piece (mp), anterior filament (af), and flagellum (f).

walls increases the surface for gamete attachment and thus for gamete production. In the testis, the greater number of plates (compared to the ovary) and the continuous covering of germinal epithelium on the plates increases the surface for production of spermatozoa. Given that male *A. echinata* (and other chiton species) release spermatozoa before egg release by females, and that the spermatozoa are likely rapidly diluted by water turbulence in the intertidal zone (Baxter & Jones, 1987; Grave, 1922, 1932; Kowalevsky, 1879; Hyman, 1967; Pearse, 1979), high production of spermatozoa may enhance fertilization success.

Earlier studies suggested that the gonad wall and tissue plates were supported by smooth muscle (Haller, 1882), by collagen fibers (Plate, 1899; Gabe & Prenant, 1949a, b), or by a combination of the two (Hyman, 1967). The latter hypothesis was indicated by the detailed transmission electron microscope studies by Selwood (1968) and also by Pearse (1979) who referred to the supporting tissue as muscular-connective tissue. We also observed both smooth muscle cells and connective fibers in the supporting tissue of *Acanthopleura echinata*. The muscle cells were evident when the tissue was stained with hematoxylin eosin, and the collagen connective fibers were indicated by a blue coloration when the tissue was stained with Mallory (Luna, 1968).

Plate (1897) indicated that the dorsal ciliated cubical epithelium of the gonad of *Acanthopleura echinata* changes in the region where the oviducts leave the ovary, first to ciliated columnar epithelium and then to glandular non-ciliated columnar epithelium. However, our observations show that the glandular non-ciliated columnar epithelium described by Plate (1897) is rather a glandular ciliated pseudostratified epithelium (Ham, 1975; Di Fiore, 1981a, b; Leeson & Leeson, 1981). The abundant secretions within this glandular epithelium suggest that this tissue provides the bulk of the materials for the mucus that covers the oocytes when they are spawned (oocytes are spawned in gelatinous strings, C. F. Gaymer, personal observation). The secretions in the slime sacs have the same characteristics as those found in the glandular cells of the oviduct wall, suggesting that the mucus of *A. echinata* is produced by the glandular cells, then stored in the slime sacs and subsequently secreted around the oocytes as they are spawned. Hyman (1967) indicates the

same function of the slime sacs for *Notoplax violacea* (Quoy & Gaimard, 1835).

Our observation that the blood sinuses in the tissue plates of the gonads of *Acanthopleura echinata* are connected to blood vessels from the dorsal aorta reveals a system that supplies nutrients and oxygen to the gonad. Previous studies suggest two main mechanisms for supplying materials to vitellogenic oocytes. The first is through follicle cells and is indicated by a decrease in the lipid and glycogen content of follicle cells during vitellogenesis in chitons and other mollusks (Raven, 1961, 1966; Nimitz & Giese, 1964). The second is via blood vessels and sinuses in the tissue plates, as described for the chiton *Sypharochiton septentriones* Ashby, 1924 [= *S. pellisserpentis* (Quoy & Gaimard, 1835)] (Selwood, 1968) and also in studies of oogenesis of insects (Kessel & Beams, 1963; Anderson, 1964; Stay, 1965). Selwood (1968) supports the hypothesis that the blood system supplies nutrients to the vitellogenic oocytes with the following observations: (1) lipid droplets first appear in the zone where the oocytes attach to the tissue plates and later appear in the rest of the cytoplasm; (2) the surface of the plasmatic membrane exposed to blood increases just prior to yolk deposition; (3) the blood connections between the vessels and tissue plates only begin to form as oogenesis is beginning; (4) micropinocytotic vesicles in the area where the oocytes attach to the tissue plates increase in number during yolk accumulation; and (5) transmission electron microscope observations do not show evidence of transfer of materials from follicle cells to vitellogenic oocytes. Our observation that there are only blood sinuses in the distal portion of the tissue plates, where the vitellogenic oocytes are attached, suggests that the blood system supplies nutrients to the vitellogenic oocytes of *A. echinata* as indicated for *S. pellisserpentis*. However, this hypothesis needs to be supported by further observations of different stages of gonad development using transmission electron microscopy and histochemistry.

In *Acanthopleura echinata,* we observed evidence of undulation of the walls of the ciliated vessels during spawning (but not of the wall of non-ciliated vessels), which suggests participation of these vessels in gamete release. The ciliated vessels may also aid in expelling the gametes during spawning in *Sypharochiton pellisserpentis,* as Selwood (1968) reports that cilia covering ciliated vessels generate currents toward the dorsal wall of the gonad, from where other cilia produce currents toward the oviducts.

In the tissue plates of the ovary of *Acanthopleura echinata,* the change from flattened cells at the base of the plates to columnar cells filled with granules at the distal edges is similar to that reported for *Sypharochiton pellisserpentis* (Selwood, 1968). Selwood indicates that the columnar cells possess lysosomes that can digest materials from the lumen (mainly degraded oocytes) by phagocytosis and micropinocytosis. Moreover, the columnar cells appear to store digested material in the form of lipid droplets and carbohydrate granules. Some of these materials are subsequently transferred via blood sinuses to developing oocytes (for yolk production) whereas non-digested materials are eliminated via the lumen to outside the ovary. Previous studies of chitons (Gabe & Prenant, 1949a, b) and oysters (Hollis, 1963) also suggest that stored substances are transferred to the ovary, but do not indicate possible mechanisms. Our observation of similar granules in the cytoplasm of the columnar cells and vitellogenic oocytes of *A. echinata* suggests that the columnar cells may supply materials to the oocytes through blood sinuses. The use of material from columnar cells would complement the supply of material from blood vessels.

The oocytes of *Acanthopleura echinata* measure 345 μm in diameter, and this exceeds the largest oocyte size previously reported for the genus *Acanthopleura* [320 μm for *A. granulata* (Gmelin, 1791), Lewis, 1960]. This is probably not related to *A. echinata* being one of the largest species of the genus *Acanthopleura* (up to ~ 14 cm in length, Boudet, 1945) as oocyte size in chitons is generally not related to adult size (Thorpe, 1962; Watanabe & Cox, 1975; Pearse, 1978, 1979; Baxter & Jones, 1987).

The chorion of *Acanthopleura echinata* is classified as type III (*sensu* Risbec, 1946) because of the numerous projections or spines. This type of chorion is also found in *Chiton cumingsi, Ischnochiton albus, Ischnochiton acomphus* Hull & Risbec, 1930, *Chaetopleura apiculata* (Say in Conrad, 1834), *Acanthopleura gemmata* (Blainville, 1825) (Schweikart, 1904; Lyngnes, 1924; Hull & Risbec, 1930–1931; Grave, 1932; review by Pearse, 1979). Chorion projections combined with mucus facilitate holding oocytes together in gelatinous masses or strings, or in attaching them to the substratum (Pearse, 1979; Eernisse, 1988; Buckland-Nicks, 1993). This could be advantageous in reducing dispersion and dilution of the oocytes in the turbulent intertidal zone where many chitons species are found (Thorpe, 1962; Pearse, 1978; Currie, 1990). The distal expansion of the projections (crown) is only found in mature oocytes of *A. echinata.* The projections are rounded, or only partly expanded, in immature oocytes (as observed in immature oocytes striped from ovaries), and expansion takes place during gamete maturation prior to spawning (as they are already as expanded in non-spawned gonads as in spontaneously spawned oocytes). Morphological changes of the structure of the chorion have also been observed in *Chaetopleura apiculata* (Buckland-Nicks, 1993) and in *Mopalia muscosa* and *Tonicella lineata* (Buckland-Nicks et al., 1988b; Buckland-Nicks, 1993). Changes in the form, size, and structure of the chorion may influence the site and mechanism of fertilization, oocyte sinking rates, adhesion and cohesion of oocytes, and brood size in brooding species (Eernisse, 1988; Buckland-Nicks, 1993). Studies on *T. lineata* and *M. muscosa* suggest that the

opening of the cupules of the chorion is caused by retraction of follicle cells on the mature oocytes (Buckland-Nicks et al., 1988b; Buckland-Nicks, 1993). This does not appear to be the case for *A. echinata* as we observed mature oocytes with expanded projections that still had follicle cells associated to them. Hyman (1967) and Anderson (1969) suggest that the follicle cells of *M. muscosa, I. albus,* and *Tonicella marmorea* (Fabricius, 1780) are responsible for chorion formation, as the endoplasmic reticulum of the follicle cells secrete a dense substance that forms a hardened rod which elongates as the follicle cell elongates. In contrast, Richter (1976) proposes that the chorion of *Lepidochitona cinerea* (Linnaeus, 1767) is secreted by the oocyte's microvilli and that follicle cells only adjust the shape of the chorion. Transmission electron microscopy studies are required to further elucidate the origin of materials forming the chorion (and the role of the follicle cells) in *A. echinata* and other chiton species.

The cytoplasm of previtellogenic oocytes in *Acanthopleura echinata* is basophilic as it is strongly stained by hematoxylin. This is explained by its intensive nuclear and nucleolar activity. The cytoplasm turns strongly basophilic due to the synthesis and storage of RNA (Selwood, 1968; Anderson, 1969; Pearse, 1979). In contrast, the cytoplasm of vitellogenic oocytes is acidophilic as it is stained with eosin. This is related to the accumulation of yolk granules and vacuoles containing mucopolysaccharides within the cytoplasm and also to the decrease in the nucleolar activity that increases acidophilic reactions (Selwood, 1968; Anderson, 1969; Pearse, 1979). Depending on the species of chiton, the oocytes are spawned either in prophase I or metaphase I of meiosis, and meiosis is completed as the polar bodies exit after fertilization (Pearse, 1979). Oocytes of *A. echinata* are spawned in prophase I of meiosis that is characterized by a large germinal vesicle (evident in sections of oviducts during spawning). Mature oocytes have staining characteristics similar to vitellogenic oocytes. Nucleolar activity is reduced to a minimum, the basophilic characteristics of cytoplasm have virtually disappeared and the cytoplasm is filled with yolk and strongly acidophilic (Selwood, 1968; Pearse, 1979). Gametogenesis in numerous chitons stops during some periods of the year (Yoshioka, 1986, 1987a, b; Pearse, 1978, 1979; Currie, 1990; Nagabhushanam & Deshpande, 1982). However, our observation of large numbers of previtellogenic and vitellogenic oocytes in *A. echinata* throughout the year indicates that oogenesis is continuous. The major seasonal change is that mature gametes are abundant in late spring to early summer and scarce during winter.

Acanthopleura echinata has "primitive" spermatozoa, characteristic of species with external fertilization (Pearse, 1979; Baccetti & Afzelius, 1976; Al-Hajj, 1987; Hodgson et al., 1988). The mid-piece containing the mitochondria is clearly distinguishable from the rest of the body and usually located posterior to the head. The anterior filament is probably an extension of the nucleus as it and the nucleus are fluorescent when stained with Acridine Orange. This also suggests the lack of a large acrosome as indicated for most chiton species (Pearse, 1979; Pearse & Woollacott, 1979; Russell-Pinto et al., 1983, 1984; Hodgson et al., 1988). Nevertheless, we cannot be sure that an acrosomic granule, as that reported for numerous chiton species (Buckland-Nicks et al., 1988a, b; Hodgson et al., 1988; Buckland-Nicks et al., 1990; Buckland-Nicks, 1993; Buckland-Nicks & Eernisse, 1993), does not exist in *A. echinata,* as we did not observe the spermatozoa using transmission electron microscropy. Sakker (1984) considered that the lack of an acrosome is a secondary condition in chitons. An acrosome is present in *Leptochiton asellus* (Gmelin, 1791), a chiton belonging to the most ancient living suborder of chitons (Pearse, 1979; Hodgson et al., 1988).

The staining of the testis also varies as spermatogenesis proceeds. The cytoplasm of spermatogonia and spermatocytes are acidophilic, strongly staining with eosin, whereas spermatids and spermatozoa are basophilic because of the nucleic acid content of most structures (Russell-Pinto et al., 1983, 1984; Al-Hajj, 1987; Hodgson et al., 1988).

Acknowledgments. We are grateful to the personnel at the Reproductive Biology Laboratory for providing the dry laboratory and histological chemicals for this study. We are also grateful to P. Penchaszadeh for his comments to improve the paper. All the experiments complied with Chilean laws.

LITERATURE CITED

Al-Hajj, H. A. 1987. Ultrastructural study of spermiogenesis in the chiton *Acanthopleura haddoni* from the Jordan Gulf of Aqaba (Red Sea). International Journal of Invertebrate Reproduction and Development 12:295–306.

Anderson, E. 1964. Oocyte differentiation and vitellogenesis in the roach *Periplaneta americana.* Journal of Cell Biology 20:131–156.

Anderson, E. 1969. Oocyte-follicle cell differentiation in two species of Aphineurans (Mollusca), *Mopalia muscosa* and *Chaetopleura apiculata.* Journal of Morphology 129:89–126.

Baccetti, B. & B. A. Afzelius. 1976. The biology of the sperm cell. Monographs in Developmental Biology 10. Karger: Basel.

Baxter, J. M. & A. M. Jones. 1987. Observation on the spawning of *Callochiton achatinus* (Brown, 1827) (Mollusca: Polyplacophora). Journal of Molluscan Studies 53:114–115.

Boudet, I. 1945. Los quitones chilenos. Revista Chilena de Historia Natural 48:122–140.

Buckland-Nicks, J. 1993. Hull cupules of chiton eggs: parachute structures and sperm focusing devices? Biological Bulletin 184:269–276.

Buckland-Nicks, J. & D. J. Eernisse. 1993. Ultraestructure of mature sperm and eggs of the brooding hermaphroditic chiton, *Lepidochitona fernaldi* Eernisse 1986, with special reference to the mechanism of fertilization. Journal of Experimental Zoology 265:567–574.

Buckland-Nicks, J., R. Koss & F. Chia. 1988a. Fertilization in a chiton: acrosome-mediated sperm-egg fusion. Gamete Research 21:199–212.

Buckland-Nicks, J., R. Koss & F. Chia. 1988b. The elusive

acrosome of chiton sperm. International Journal of Invertebrate Reproduction and Development 13:193–198.

Buckland-Nicks, J., R. Koss & F. Chia. 1990. Spermiogenesis in Polyplacophora, with special reference to acrosome formation (Mollusca). Zoomorphology 109:179–188.

Christiansen, M. E. 1954. The life history of *Lepidopleurus asellus* (Spengler) (Placophora). Nytt Magasin for Zoologi 2:52–72.

Currie, D. R. 1990. Reproductive periodicity of three species of chitons at a site in New South Wales, Australia (Mollusca: Polyplacophora). Invertebrate Reproduction and Development 17:25–32.

Di Fiore, M. 1981a. Diagnóstico histológico. Tomo I. El Ateneo: Buenos Aires, Argentina. 452 pp.

Di Fiore, M. 1981b. Diagnóstico histológico. Tomo II. El Ateneo: Buenos Aires, Argentina. 229 pp.

Dohmen, M. R. 1983. Gametogenesis. Pp. 1–48 in N. H. Verdonk, J. A. M. van den Biggelaar & A. S. Tompa (eds.), The Mollusca. Vol 3. Development. Academic Press: New York.

Eernisse, D. J. 1988. Reproductive patterns in six species of *Lepidochitona* (Molluca: Polyplacophora) from the Pacific Coast of North America. Biological Bulletin 174:287–302.

Gabe, M. & M. Prenant. 1949a. Contribution à l'histologie de l'ovogénèse chez les polyplacophores. Cellule 53:99–117.

Gabe, M. & M. Prenant. 1949b. Données histologiques sur le tissu conjunctif des polyplacophores. Archives of Anatomy and Microscopy 38:65–75.

Grave, B. H. 1922. An analysis of the spawning habits and spawning stimuli of *Chaetopleura apiculata* (Say). Biological Bulletin 42:234–256.

Grave, B. H. 1932. Embryology and life history of *Chaetopleura apiculata*. Journal of Morphology 54:153–160.

Guiller, E. 1959. Intertidal belt-forming species on the rocky coasts of northern Chile. Papers and Proceedings of the Royal Society of Tasmania 93:33–59.

Haller, B. 1882. Die organisation der chitonen der Adria Arb. Zool. Inst. Univ. Wien. 4:1–74.

Ham, A. 1975. Tratado de histología. Interamericana: Mexico. 935 pp.

Hodgson, A. N., J. M. Baxter, M. G. Sturrock & R. T. F. Bernard. 1988. Comparative spermatology of 11 species of Polyplacophora (Mollusca) from the suborders Lepidopleurina, Chitonina and Acanthochitonina. Proceedings of the Royal Society of London B 235:161–177.

Hollis, P. J. 1963. Some studies on the New Zealand oysters. Zoology Publication of Victoria University of Wellington 31:1–28.

Hull, A. F. B. & J. Risbec. 1930–1931. The loricates of the New Caledonian region (class Mollusca-Order Loricata). Australian Zoologist 6:277–286, 372–386.

Hyman, L. H. 1967. The Invertebrates: Mollusca 1. Vol VI. McGraw-Hill: USA. 792 pp.

Kessel, R. G. & H. W. Beams. 1963. Micropinocytes and yolk formation in oocytes of the small milkweed bug. Expl?? in Cellular Research 30:440–459.

Kowalevsky, A. 1879. Ueber der Entwickelung der chitonen. Zoologischer Anzeiger 2:469–473.

Leeson, C. & T. Leeson. 1981. Histología. Interamericana: Mexico. 564 pp.

Leloup, E. 1956. Polyplacophora. Reports of the Lund University Chile Expedition (1948–1949) 27:1–93.

Lewis, J. B. 1960. The fauna of rocky shores of Barbados, West Indies. Canadian Journal of Zoology 38:391–435.

Luna, L. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill: USA. 258 pp.

Lyngnes, R. 1924. Zur Kenntnis der Eihüllen der Chitonen. Skrifter Videnskabers Selskab Kristiania 20:1–19.

Nagabhushanam, R. & U. D. Deshpande. 1982. Reproductive cycle of the chiton *Chiton iatricus* and environmental control of its gonad growth. Marine Biology 67:9–13.

Nimitz, M. A. & A. C. Giese. 1964. Histochemical changes correlated with reproductive activity and nutrition in the chiton *Katharina tunicata*. Quarterly Journal of Microscopic Science 105:481–495.

Osorio, C. 1989. Las pesquerías de moluscos en el Pacífico Sudeste. Revista Pacífico Sur (Número especial):529–542.

Otaíza, R. 1986. Patrones de distribución vertical de chitones y efecto de pastoreo de *Chiton granosus* en roqueríos intermareales de Chile central. Monografías Biológicas 4:173–190.

Otaíza, R. & B. Santelices. 1985. Vertical distribution of chitons (Mollusca: Polyplacophora) in the rocky intertidal zone of central Chile. Journal of Experimental Marine Biology and Ecology 86:229–240.

Pashchenko, S. V. & A. L. Drozdov. 1998. Morphology of gametes in five species of far-eastern chitons. Invertebrate Reproduction and Development 33:47–56.

Pearse, J. S. 1978. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. IV. The chitons *Acanthopleura haddoni* Winckworth and *Onithochiton lyelli* (Sowerby), and the abalone *Haliotis pustulata* Reeve. Bulletin of Marine Science 28:92–101.

Pearse, J. S. 1979. Polyplacophora. Pp. 27–85 in A. C. Giese & J. S. Pearse (eds.), Reproduction in Marine Invertebrates. Vol. 5. Molluscs: Pelecypods and Lesser Classes. Academic Press: New York.

Pearse, J. S. & R. M. Woollacott. 1979. Chiton sperm: no acrosome? American Zoologist 19:956–956.

Plate, L. 1897. Die anatomie und phylogenie der chitonen. Zoologischer Anzeiger 4:(Suppl) 1–243.

Plate, L. 1899. Die anatomie und phylogenie der chitonen. Zoologischer Anzeiger 5:(Suppl) 15–216.

Raven, C. P. 1961. Oogenesis: The Storage of Developmental Information. Pergamon Press: New York. 274 pp.

Raven, C. P. 1966. Morphogenesis: The Analysis of Molluscan Development. 2nd ed. Pergamon Press: Oxford. 365 pp.

Richter, H. P. 1976. Feinstrukturelle untersuchungen zur oogenese der Käferschnecke *Lepidochitona cinereus* (Mollusca, Polyplacophora). Helgoländer Meeresuntersuchungen 28: 250–303.

Risbec, J. 1946. Études anatomiques sur les amphineures de la Nouvelle-Calédonie. Journal de la Societé des Océanistes 2: 129–190.

Russell-Pinto, F., C. Azevedo & T. Barandela. 1983. Fine structure of the spermatozoa of *Chiton marginatus* (Mollusca: Amphineura), with special reference to nucleus maturation. Gamete Research 8:345–355.

Russell-Pinto, F., C. Azevedo & E. Oliveira. 1984. Comparative ultastructural studies of spermiogenesis and spermatozoa in some species of Polyplacophora (Mollusca). International Journal of Invertebrate Reproduction and Development 7:267–277.

Sakker, E. R. 1984. Sperm morphology, spermatogenesis and spermiogenesis of three species of chiton (Mollusca, Polyplacophora). Zoomorphology 104:111–124.

Santelices, B., J. Vásquez & I. Meneses. 1986. Patrones de

distribución de un gremio de moluscos herbívoros en hábitats intermareales expuestos de Chile central. Monografías Biológicas 4:147–171.

Schweikart, A. 1904. Die bildung der eihüllen und ihrer anhänge bei den chitonen. Zoologischer Anzeiger 27:636–648.

Selwood, L. 1968. Interrelationships between developing oocytes and ovarian tissues in the chiton *Sypharochiton septentriones* (Ashby) (Mollusca, Polyplacophora). Journal of Morphology 125:71–104.

Sernapesca. 2000. Anuario Estadístico de Pesca. Sernapesca, Ministerio de Economía, Fomento y Reconstrucción, Chile.

Stay, B. 1965. Protein uptake in the oocytes of the Cecropea moth. Journal of Cell Biology 26:49–62.

Thorpe, S. R. 1962. A preliminary report on spawning and related phenomena in California chitons. The Veliger 4:202–210.

Watanabe, J. M. & L. R. Cox. 1975. Spawning behavior and larval development in *Mopalia lignosa* and *Mopalia muscosa* (Mollusca: Polyplacophora) in central California. The Veliger 18:(Suppl) 18–27.

Yoshioka, E. 1986. Histological studies on the gonad and gametogenesis of the chiton *Acanthopleura japonica* (Lischke). Venus 45:109–115.

Yoshioka, E. 1987a. Environmental cue to initiate gametogenesis in the chiton *Acanthopleura japonica.* Venus 46:173–177.

Yoshioka, E. 1987b. Annual reproductive cycle of the chiton *Acanthopleura japonica.* Marine Biology 96:371–374.